09/700712

FIG. 1

SEQ ID NO: 1:

```
GAGAAGGTTT GTTATGCCTC AGGGTTATCT GCAGTTTCCC AATATTGACC CCGTATTGTT   60
TTCGATCGGC CCTCTAGCGG TGCGCTGGTA TGGCTTGATG TATTTGGTGG GTTTCCTTTT  120
TGCTATGTGG TTGGCCAATC GCCGAGCGGA TCGCGCGGGC AGTGGTTGGA CGCGTGAGCA  180
AGTCTCTGAC TTGTTATTCG CCGGCTTTTT AGGTGTAGTG ATCGGTGGCC GAGTTGGTTA  240
TGTGATCTTC TACAATTTTG ATCTGTTCCT TGCTGACCCT CTTTATTTAT TCAAAGTGTG  300
GACTGGCGGC ATGTCCTTCC ACGGCGGCTT ATTGGGTGTG ATCACCGCCA TGTTCTGGTA  360
TGCGCGTAAA AACCAACGCA CCTTCTTTGG TGTGGCCGAT TTTGTTGCCC CTTTAGTGCC  420
ATTCGGTTTG GGGATGGGAC GTATCGGTAA CTTTATGAAT AGTGAACTTT GGGGACGAGT  480
AACGGATGTG CCTTGGGCTT TTGTATTCCC TAATGGTGGC CCACTGCCGC GCCATCCTTC  540
ACAGCTTTAT GAATTCGCCT TAGAAGGCGT GGTTCTGTTC TTTATTCTTA ATTGGTTTAT  600
TGGTAAACCT CGTCCGCTAG GCAGCGTATC CGGACTGTTT TTAGCTGGAT ACGGTACATT  660
CCGCTTCCTT GTGGAATACG TCCGTGAGCC AGATGCTCAG TTGGGTCTGT TTGGTGGCTT  720
CATTTCAATG GGGCAAATCC TCTCCTTACC TATGGTGATC ATCGGTATTT TGATGATGGT  780
TTGGTCTTAC AAGCGCGGTT TGTATCAAGA CCGTGTAGCA GCAAAATAGG GTAGTTAGGT  840
GAAACAGTAT TTAGATCTTT GTCAGCGCAT CGTCGATCAA GGTGTTTGGG TTGAAAATGA  900
ACGAACGGGC AAGCGTTGTT TGACTGTGAT TAATGCCGAT TTGACCTACG ATGTGGGCAA  960
CAATCAGTTT CCTCTAGTGA CTACACGCAA GAGTTTTTGG AAAGCTGCCG TAGCCGAGTT 1020
GCTCGGCTAT ATTCGTGGTT ACGATAATGC GGCGGATTTT CGCCAATTAG GTACCAAAAC 1080
CTGGGATGCT AATGCCAATT TAAACCAAGC ATGGCTCAAC AATCCTTACC GTAAAGGTGA 1140
GGATGACATG GGACGCGTGT ATGGTGTTCA GGGTAGAGCT TGGGCTAAGC CTGATGGTGG 1200
TCATATTGAC CAGTTGAAAA AGATTGTTGA TGATTTGAGC CGTGGCGTTG ATGACCGAGG 1260
TGAAATTCTT AACTTCTACA ATCCGGGTGA ATTTCACATG GGGTGTTTGC GCCCTTGCAT 1320
GTACAGCCAT CATTTTTCAT TGCTGGGGGA TACCTTGTAT CTCAACAGTA CTCAGCGTTC 1380
ATGTGATGTG CCCTTGGGGT TGAATTTCAA CATGGTGCAG GTTTATGTGT TCCTTGCGCT 1440
GATGGCACAG ATCACAGGGA AAAAGCCGGG CTTGGCGTAT CACAAGATCG TCAATGCGCA 1500
```

FIG.1 (cont.)

CATTTACCAA GATCAACTCG AATTGATGCG CGATGTGCAG CTAAAACGTG AGCCATTCCC 1560

AGCGCCTCAG TTCCATATCA ATCCAAAGAT TAAAACACTG CAGGATTTGG AAACTTGGGT 1620

CACTTTGGAT GATTTTGACG TCACCGGATA TCAGTTCCAC GATCCTATTC AATACCCGTT 1680

TTCAGTCTAA TCCCGTATTC AGGCGGTATG GCTTGATGGG TTTTATATAA AAAAAGCTCC 1740

CGAAGGTCGG GAGCTTTTTT TATACAGATG ATGCTTTAAC GCTTAAGCGG TTAGGGCAAG 1800

AATGCTGCCG GGGATGACGA CAAACACACC CAATAAGTAA CTCACCACCA CCATTTTGCT 1860

CTTACAAGCC CAAGTTGAGA TGAGCTCAGC ACCTTTAATA GGCAGTTCGC GTAAGAAAGG 1920

AATACCGTAA ATCAAGACCG TAGCCATCAA GTTAAAGCTT AAGTGCACCA GCGCAATTTG 1980

CAGAGCAAAC ACGGCAAACT CACCAGAGAC AGCGGTTGCG GCGAGCAGAG CAGTAATACA 2040

AGTGCCAATG TTCGCACCTA AGGTAAATGG GTAGATTTCA CGCACTTTCA GCACGCCAGA 2100

GCCCACGAGA GGAACCATTA GGCTGGTTGT GGTCGATGAA GATTGAACTA ATACCGTAAC 2160

CACTGTACCT GAAGCAATAC CGTGTAGTGG GCCTCGGCCA ATCGCATTTT GTAGAATTTC 2220

ACGTGCGCGG CCAACCATCA AACTCTTCAT CAGTTTGCCC ATCACCGTAA TGGCGACGAA 2280

AATGGTCGCA ATACCCAATA CGATAAGTGC GACACCACCG AAAGTATTAC CCAATACCGA 2340

AAGCTGGGTT TCAAGCCCTG TGATGACAGG TTTGGTAATC GGTTTGATAA AATCAAAACC 2400

TTTCATGCTC ATATCGCCAG TCGCAAGCAG AGGCGAAACG AGCCAGTGTG AGACTTTCTC 2460

TAAAATGCCA AACATCATTT CTAGAGGTAG GAAGATCAGC ACCGCGAGAA GATTGAAAAA 2520

ATCGTGGATG GTGGCACTGG CGAAAGCACG GCGAAACTCT TCTTTACAGC GCATATGGCC 2580

AAGGCTGACG AGAGTATTGG TCACAGTAGT ACCAATATTG GCACCCATCA CCATAGGAAT 2640

CGCGGTTTCA ACCGGTAACC CACCGGCAAC GAGACCAACA ATAATAGAAG TCACCGTGCT 2700

TGAGGATTGA ATCAGTGCCG TTGCCACTAA ACCAATCATC AATCCTGCAA TTGGGTGGGA 2760

AGCAAATTCA AATAGAACTT TGGCTTGATC GCCGGTTGCC CATTTAAAAC CGCTGCCGAC 2820

CATCGCGACT GCAAGAAGTA GTAAATACAG CATGAAAGCC AAGTTTGCCC AACGTAGGCC 2880

TTTCGTGGTC AGCGAAATCG GCGCTGCAG 2909

FIG.2

SEQ ID NO: 2:

```
GAGAAGGTTT GTTATGCCTC AGGGTTATCT GCAGTTTCCC AATATTGACC CCGTATTGTT   60
TTCGATCGGC CCTCTAGCGG TGCGCTGGTA TGGCTTGATG TATTTGGTGG GTTTCCTTTT  120
TGCTATGTGG TTGGCCAATC GCCGAGCGGA TCGCGCGGGC AGTGGTTGGA CGCGTGAGCA  180
AGTCTCTGAC TTGTTATTCG CCGGCTTTTT AGGTGTAGTG ATCGGTGGCC GAGTTGGTTA  240
TGTGATCTTC TACAATTTTG ATCTGTTCCT TGCTGACCCT CTTTATTTAT TCAAAGTGTG  300
GACTGGCGGC ATGTCCTTCC ACGGCGGCTT ATTGGGTGTG ATCACCGCCA TGTTCTGGTA  360
TGCGCGTAAA AACCAACGCA CCTTCTTTGG TGTGGCCGAT TTTGTTGCCC CTTTAGTGCC  420
ATTCGGTTTG GGGATGGGAC GTATCGGTAA CTTTATGAAT AGTGAACTTT GGGGACGAGT  480
AACGGATGTG CCTTGGGCTT TTGTATTCCC TAATGGTGGC CCACTGCCGC GCCATCCTTC  540
ACAGCTTTAT GAATTCGCCT TAGAAGGCGT GGTTCTGTTC TTTATTCTTA ATTGGTTTAT  600
TGGTAAACCT CGTCCGCTAG GCAGCGTATC CGGACTGTTT TTAGCTGGAT ACGGTACATT  660
CCGCTTCCTT GTGGAATACG TCCGTGAGCC AGATGCTCAG TTGGGTCTGT TTGGTGGCTT  720
CATTTCAATG GGGCAAATCC TCTCCTTACC TATGGTGATC ATCGGTATTT TGATGATGGT  780
TTGGTCTTAC AAGCGCGGTT TGTATCAAGA CCGTGTAGCA GCAAAATAGG GTAGTTAG    838
```

FIG.3

SEQ ID NO: 3:

```
TAATCCCGTA TTCAGGCGGT ATGGCTTGAT GGGTTTTATA TAAAAAAAGC TCCCGAAGGT   60
CGGGAGCTTT TTTTATACAG ATGATGCTTT AACGCTTAAG CGGTTAGGGC AAGAATGCTG  120
CCGGGGATGA CGACAAACAC ACCCAATAAG TAACTCACCA CCACCATTTT GCTCTTACAA  180
GCCCAAGTTG AGATGAGCTC AGCACCTTTA ATAGGCAGTT CGCGTAAGAA AGGAATACCG  240
TAAATCAAGA CCGTAGCCAT CAAGTTAAAG CTTAAGTGCA CCAGCGCAAT TTGCAGAGCA  300
AACACGGCAA ACTCACCAGA GACAGCGGTT GCGGCGAGCA GAGCAGTAAT ACAAGTGCCA  360
ATGTTCGCAC CTAAGGTAAA TGGGTAGATT TCACGCACTT TCAGCACGCC AGAGCCCACG  420
AGAGGAACCA TTAGGCTGGT TGTGGTCGAT GAAGATTGAA CTAATACCGT AACCACTGTA  480
CCTGAAGCAA TACCGTGTAG TGGGCCTCGG CCAATCGCAT TTTGTAGAAT TTCACGTGCG  540
CGGCCAACCA TCAAACTCTT CATCAGTTTG CCCATCACCG TAATGGCGAC GAAAATGGTC  600
GCAATACCCA ATACGATAAG TGCGACACCA CCGAAAGTAT TACCCAATAC CGAAAGCTGG  660
GTTTCAAGCC CTGTGATGAC AGGTTTGGTA ATCGGTTTGA TAAAATCAAA ACCTTTCATG  720
CTCATATCGC CAGTCGCAAG CAGAGGCGAA ACGAGCCAGT GTGAGACTTT CTCTAAAATG  780
CCAAACATCA TTTCTAGAGG TAGGAAGATC AGCACCGCGA GAAGATTGAA AAAATCGTGG  840
ATGGTGGCAC TGGCGAAAGC ACGGCGAAAC TCTTCTTTAC AGCGCATATG GCCAAGGCTG  900
ACGAGAGTAT TGGTCACAGT AGTACCAATA TTGGCACCCA TCACCATAGG AATCGCGGTT  960
TCAACCGGTA ACCCACCGGC AACGAGACCA ACAATAATAG AAGTCACCGT GCTTGAGGAT 1020
TGAATCAGTG CCGTTGCCAC TAAACCAATC ATCAATCCTG CAATTGGGTG GGAAGCAAAT 1080
TCAAATAGAA CTTTGGCTTG ATCGCCGGTT GCCCATTTAA AACCGCTGCC GACCATCGCG 1140
ACTGCAAGAA GTAGTAAATA CAGCATGAAA GCCAAGTTTG CCCAACGTAG GCCTTTCGTG 1200
GTCAGCGAAA TCGGCGCTGC AG                                         1222
```

09/700712

FIG.4

SEQ ID NO: 4:

Val	Lys	Gln	Tyr	Leu	Asp	Leu	Cys	Gln	Arg	Ile	Val	Asp	Gln	Gly	Val
1				5					10					15	
Trp	Val	Glu	Asn	Glu	Arg	Thr	Gly	Lys	Arg	Cys	Leu	Thr	Val	Ile	Asn
			20					25					30		
Ala	Asp	Leu	Thr	Tyr	Asp	Val	Gly	Asn	Asn	Gln	Phe	Pro	Leu	Val	Thr
		35					40					45			
Thr	Arg	Lys	Ser	Phe	Trp	Lys	Ala	Ala	Val	Ala	Glu	Leu	Leu	Gly	Tyr
	50					55					60				
Ile	Arg	Gly	Tyr	Asp	Asn	Ala	Ala	Asp	Phe	Arg	Gln	Leu	Gly	Thr	Lys
65					70					75					80
Thr	Trp	Asp	Ala	Asn	Ala	Asn	Leu	Asn	Gln	Ala	Trp	Leu	Asn	Asn	Pro
				85					90					95	
Tyr	Arg	Lys	Gly	Glu	Asp	Asp	Met	Gly	Arg	Val	Tyr	Gly	Val	Gln	Gly
			100					105					110		
Arg	Ala	Trp	Ala	Lys	Pro	Asp	Gly	Gly	His	Ile	Asp	Gln	Leu	Lys	Lys
		115					120					125			
Ile	Val	Asp	Asp	Leu	Ser	Arg	Gly	Val	Asp	Asp	Arg	Gly	Glu	Ile	Leu
	130					135					140				
Asn	Phe	Tyr	Asn	Pro	Gly	Glu	Phe	His	Met	Gly	Cys	Leu	Arg	Pro	Cys
145					150					155					160
Met	Tyr	Ser	His	His	Phe	Ser	Leu	Leu	Gly	Asp	Thr	Leu	Tyr	Leu	Asn
				165					170					175	
Ser	Thr	Gln	Arg	Ser	Cys	Asp	Val	Pro	Leu	Gly	Leu	Asn	Phe	Asn	Met
			180					185					190		
Val	Gln	Val	Tyr	Val	Phe	Leu	Ala	Leu	Met	Ala	Gln	Ile	Thr	Gly	Lys
		195					200					205			
Lys	Pro	Gly	Leu	Ala	Tyr	His	Lys	Ile	Val	Asn	Ala	His	Ile	Tyr	Gln
	210					215					220				
Asp	Gln	Leu	Glu	Leu	Met	Arg	Asp	Val	Gln	Leu	Lys	Arg	Glu	Pro	Phe
225					230					235					240
Pro	Ala	Pro	Gln	Phe	His	Ile	Asn	Pro	Lys	Ile	Lys	Thr	Leu	Gln	Asp
				245					250					255	
Leu	Glu	Thr	Trp	Val	Thr	Leu	Asp	Asp	Phe	Asp	Val	Thr	Gly	Tyr	Gln
			260					265					270		
Phe	His	Asp	Pro	Ile	Gln	Tyr	Pro	Phe	Ser	Val					
		275					280								

09/200712

FIG. 5

SEQ ID NO: 5:

Met	Pro	Gln	Gly	Tyr	Leu	Gln	Phe	Pro	Asn	Ile	Asp	Pro	Val	Leu	Phe
1				5					10					15	
Ser	Ile	Gly	Pro	Leu	Ala	Val	Arg	Trp	Tyr	Gly	Leu	Met	Tyr	Leu	Val
			20					25					30		
Gly	Phe	Leu	Phe	Ala	Met	Trp	Leu	Ala	Asn	Arg	Arg	Ala	Asp	Arg	Ala
		35					40					45			
Gly	Ser	Gly	Trp	Thr	Arg	Glu	Gln	Val	Ser	Asp	Leu	Leu	Phe	Ala	Gly
	50					55					60				
Phe	Leu	Gly	Val	Val	Ile	Gly	Gly	Arg	Val	Gly	Tyr	Val	Ile	Phe	Tyr
65					70					75					80
Asn	Phe	Asp	Leu	Phe	Leu	Ala	Asp	Pro	Leu	Tyr	Leu	Phe	Lys	Val	Trp
				85					90					95	
Thr	Gly	Gly	Met	Ser	Phe	His	Gly	Gly	Leu	Leu	Gly	Val	Ile	Thr	Ala
			100					105					110		
Met	Phe	Trp	Tyr	Ala	Arg	Lys	Asn	Gln	Arg	Thr	Phe	Phe	Gly	Val	Ala
		115					120					125			
Asp	Phe	Val	Ala	Pro	Leu	Val	Pro	Phe	Gly	Leu	Gly	Met	Gly	Arg	Ile
	130					135					140				
Gly	Asn	Phe	Met	Asn	Ser	Glu	Leu	Trp	Gly	Arg	Val	Thr	Asp	Val	Pro
145					150					155					160
Trp	Ala	Phe	Val	Phe	Pro	Asn	Gly	Gly	Pro	Leu	Pro	Arg	His	Pro	Ser
				165					170					175	
Gln	Leu	Tyr	Glu	Phe	Ala	Leu	Glu	Gly	Val	Val	Leu	Phe	Phe	Ile	Leu
			180					185					190		
Asn	Trp	Phe	Ile	Gly	Lys	Pro	Arg	Pro	Leu	Gly	Ser	Val	Ser	Gly	Leu
		195					200					205			
Phe	Leu	Ala	Gly	Tyr	Gly	Thr	Phe	Arg	Phe	Leu	Val	Glu	Tyr	Val	Arg
	210					215					220				
Glu	Pro	Asp	Ala	Gln	Leu	Gly	Leu	Phe	Gly	Gly	Phe	Ile	Ser	Met	Gly
225					230					235					240
Gln	Ile	Leu	Ser	Leu	Pro	Met	Val	Ile	Ile	Gly	Ile	Leu	Met	Met	Val
				245					250					255	
Trp	Ser	Tyr	Lys	Arg	Gly	Leu	Tyr	Gln	Asp	Arg	Val	Ala	Ala	Lys	
			260					265					270		

FIG. 6

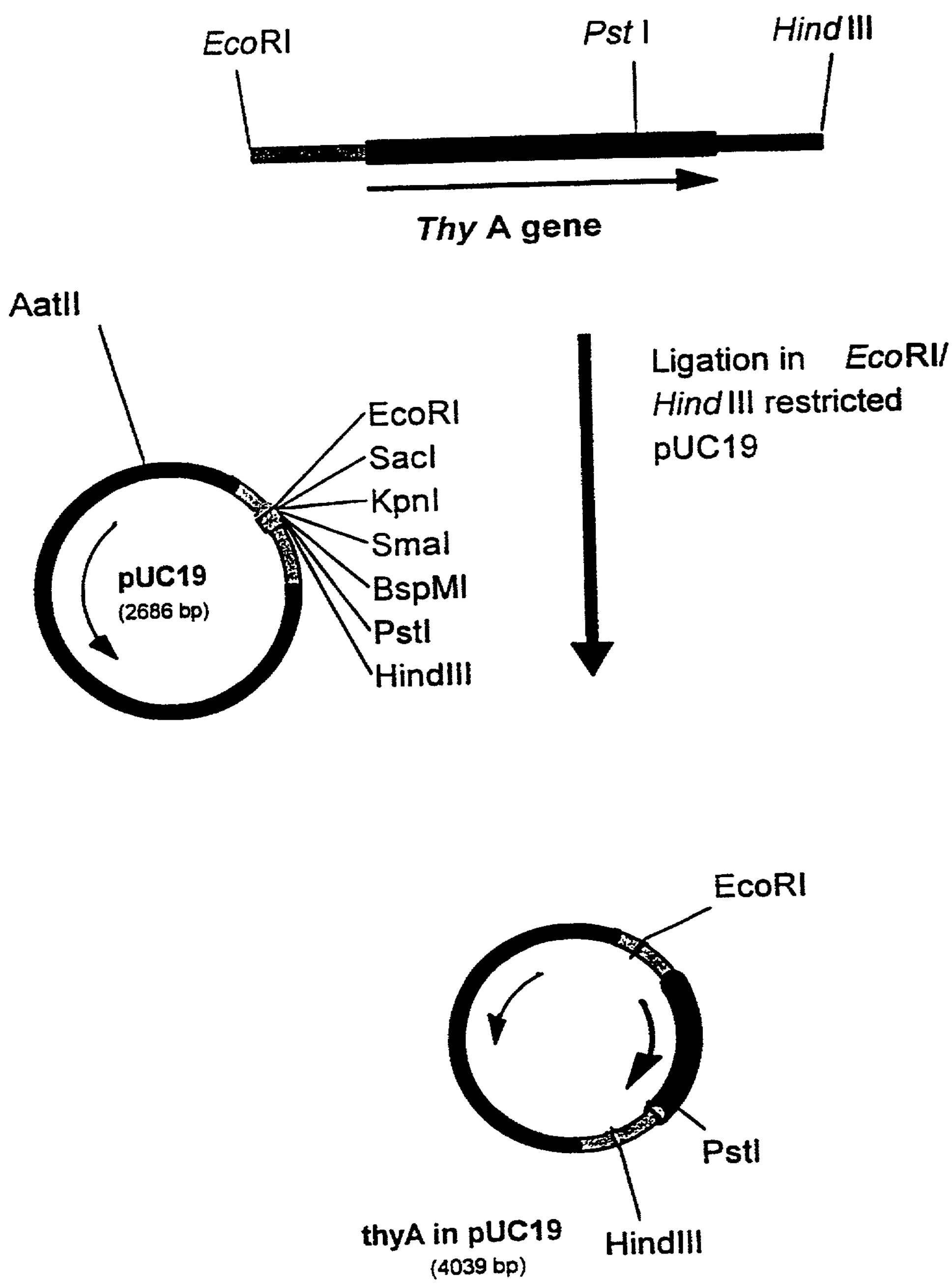

EcoRI
Pst I
Hind III
Thy A gene
AatII
EcoRI
SacI
KpnI
SmaI
BspMI
PstI
HindIII
pUC19
(2686 bp)
Ligation in EcoRI/ Hind III restricted pUC19
EcoRI
PstI
HindIII
thyA in pUC19
(4039 bp)

FIG. 7

```
E. coli     : MKQYLELMQKVLDEGT-QKNDRTGTGTLSIFGHQMRFNL-QDGFPLVTTKRCHLRSIIHE
              :||||:| |:::|:|:  :|:|||:  |:::: :: ::: :: ||||||:::  :: : |
V. cholerae : VKQYLDLCQRIVDQGVWVENERTGKRCLTVINADLTYDVGNNQFPLVTTRKSFWKAAVAE
              :||||:||:|||::| ||:||||||:|||||||||:|||:||||||:|||||:||||:||
H. influenza: MKQYLELCRRIVSEGEWVANERTGKHCLTVINADLEYDVANNQFPLITTRKSYWKAAIAE

E. coli     : LLWFLQGDTNIAYHENNVTIWD--------EWADE-----NGDLGPVYGKQWRAWPTPDG
              || :::| :| | ::: :::||        :| ::     ::|:|:||| | |||::|||
V. cholerae : LLGYIRGYDNAADFRQLGTKTWDANANLNQAWLNNPYRKGEDDMGRVYGVQGRAWAKPDG
              :||||||||||||||:||||||||||| |:|||:||:|:| |||||||||||||| ||:|
H. influenza: FLGYIRGYDNAADFRALGTKTWDANANENAAWLANPHRRGVDDMGRVYGVQGRAWRKPNG

E. coli     : RHIDQITTVLNQLKNDPDSRRIIVSAWNVGELDKMALAPCHAFFQFYVADGKLSCQLYQR
               ||||:::::::|::: |:|  |:: :| ||:::  | ||    :| : :::|  :  ||
V. cholerae : GHIDQLKKIVDDLSRGVDDRGEILNFYNPGEFHMGCLRPCMYSHHFSLLGDTLYLNSTQR
              : ||||:|||::|::|:|||||||:|:|||||::|||||||::| |||:||||:|:| ||
H. influenza: ETIDQLRKIVNNLTKGIDDRGEILTFFNPGEFDLGCLRPCMHTHTFSLVGDTLHLTSYQR

E. coli     : SCDVFLGLPFNIASYALLVHMMAQQCDLEVGDFVWTGGDTHLYSNHMD-QTHLQLSREPR
              |||| ||| ||::   ::: :|||  : : |    :  ::|:| ::::   ::||:|||
V. cholerae : SCDVPLGLNFNMVQVYVFLALMAQITGKKPGLAYHKIVNAHIYQDQLELMRDVQLKREPF
              ||||||||||| :||::||||||||||||:| |||||||||||:||||||||||||||||
H. influenza: SCDVPLGLNFNQIQVFTFLALMAQITGKKAGKAYHKIVNAHIYEDQLELMRDVQLKREPF

E. coli     : PLPKLIIKRKPESIFDY----RFEDFEIEGYDPHPGIKAPVAI
              | |:: |::| ::: |      ::||:::||: |  |: | ::
V. cholerae : PAPQFHINPKIKTLQDLETWVTLDDFDVTGYQFHDPIQYPFSV
              | |:::|||||||||||||||||||||||||||||||||||||
H. influenza: PLPKELINPDIKTLEDLETWVTMDDFKVVGYQSHEPIKYPFSV
```

FIG. 8

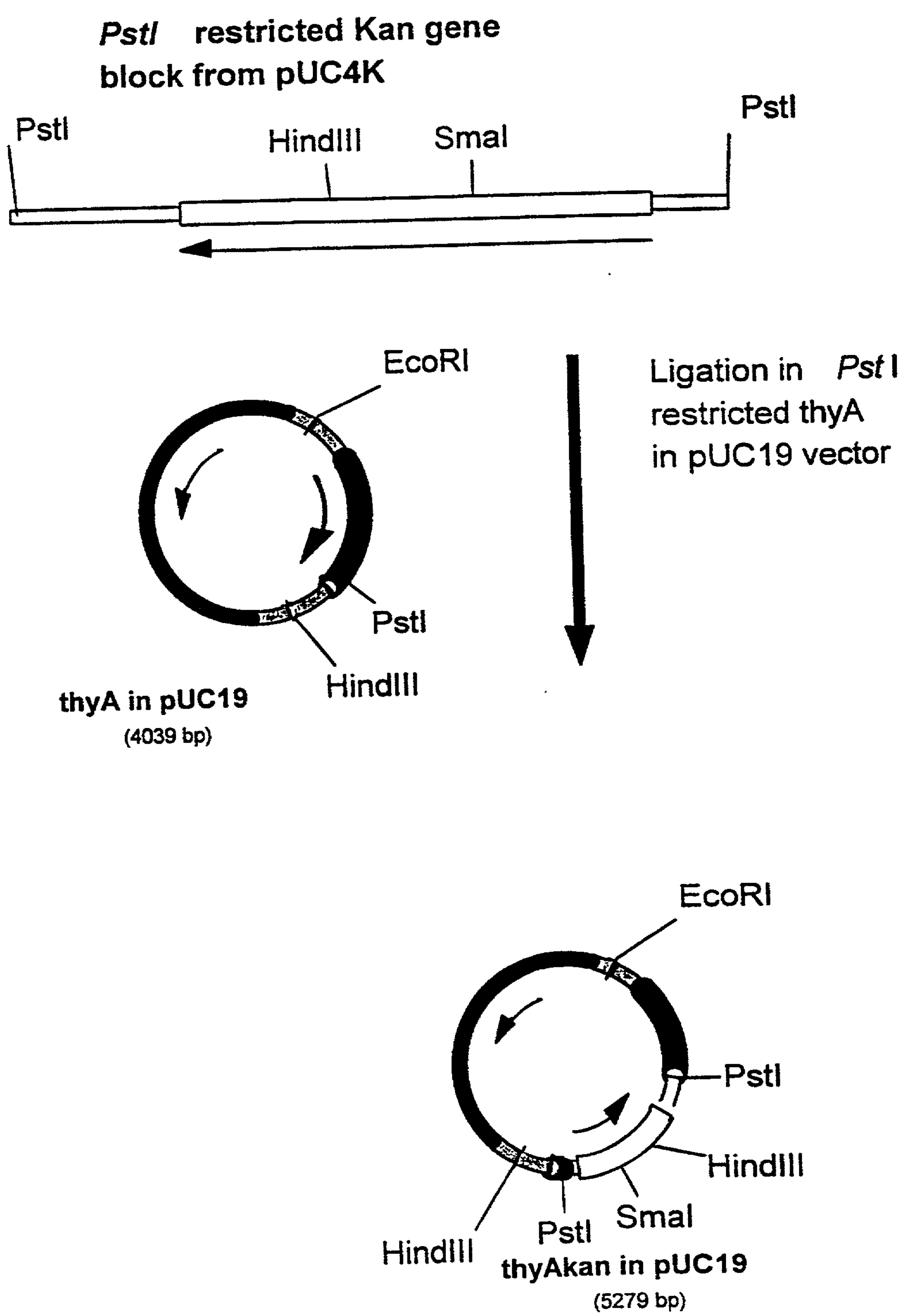
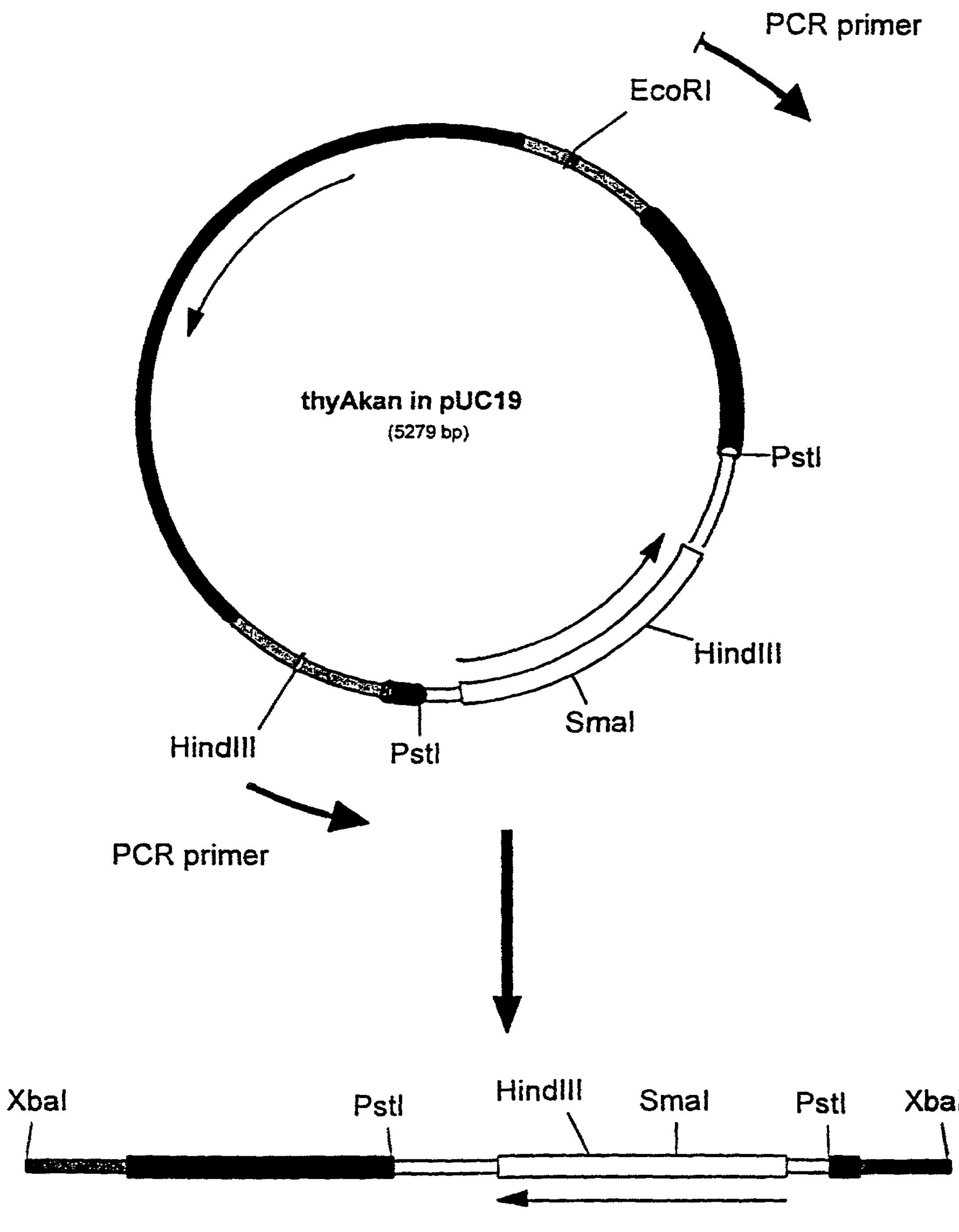

PstI restricted Kan gene
block from pUC4K
PstI
HindIII
SmaI
PstI
EcoRI
Ligation in *Pst* I
restricted thyA
in pUC19 vector
PstI
HindIII
thyA in pUC19
(4039 bp)
EcoRI
PstI
HindIII
SmaI
PstI
HindIII
thyAkan in pUC19
(5279 bp)

FIG. 9

PCR to generate thyA-Kan-thyA fragment with *Xba*I ends. Primers were choosen so that the *Eco*RI and *Hind*III sites were eliminated



thyA with kan XbaI ends
(2643 bp)

FIG. 10

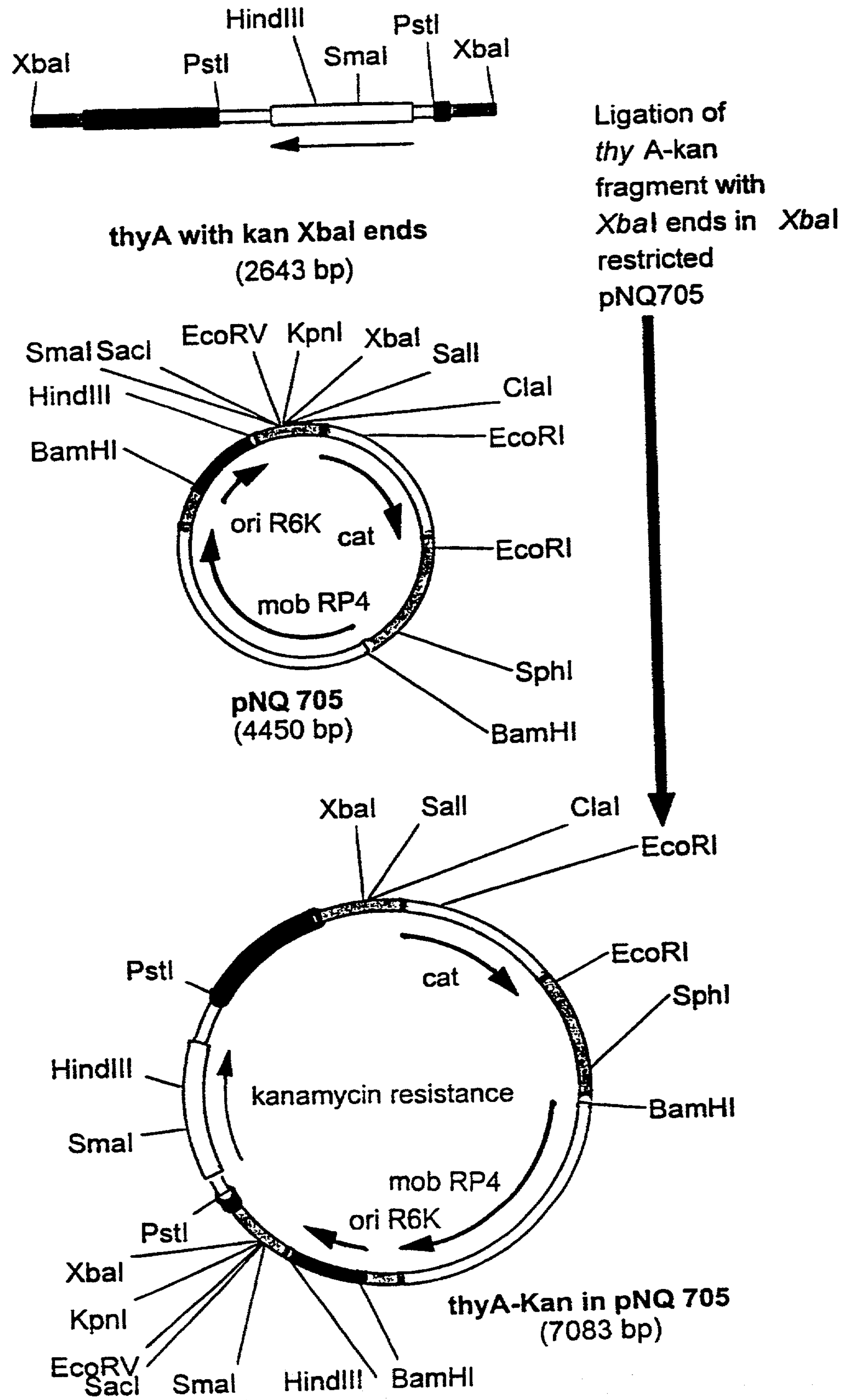

XbaI
PstI
HindIII
SmaI
PstI
XbaI
thyA with kan XbaI ends
(2643 bp)
Ligation of
thy A-kan
fragment with
*Xba*I ends in *Xba*I
restricted
pNQ705
SmaI SacI
EcoRV
KpnI
XbaI
SalI
ClaI
HindIII
EcoRI
BamHI
ori R6K
cat
EcoRI
mob RP4
SphI
pNQ 705
(4450 bp)
BamHI
XbaI
SalI
ClaI
EcoRI
EcoRI
cat
PstI
SphI
HindIII
kanamycin resistance
BamHI
SmaI
mob RP4
ori R6K
PstI
XbaI
KpnI
EcoRV
SacI
SmaI
HindIII
BamHI
thyA-Kan in pNQ 705
(7083 bp)

FIG. 11

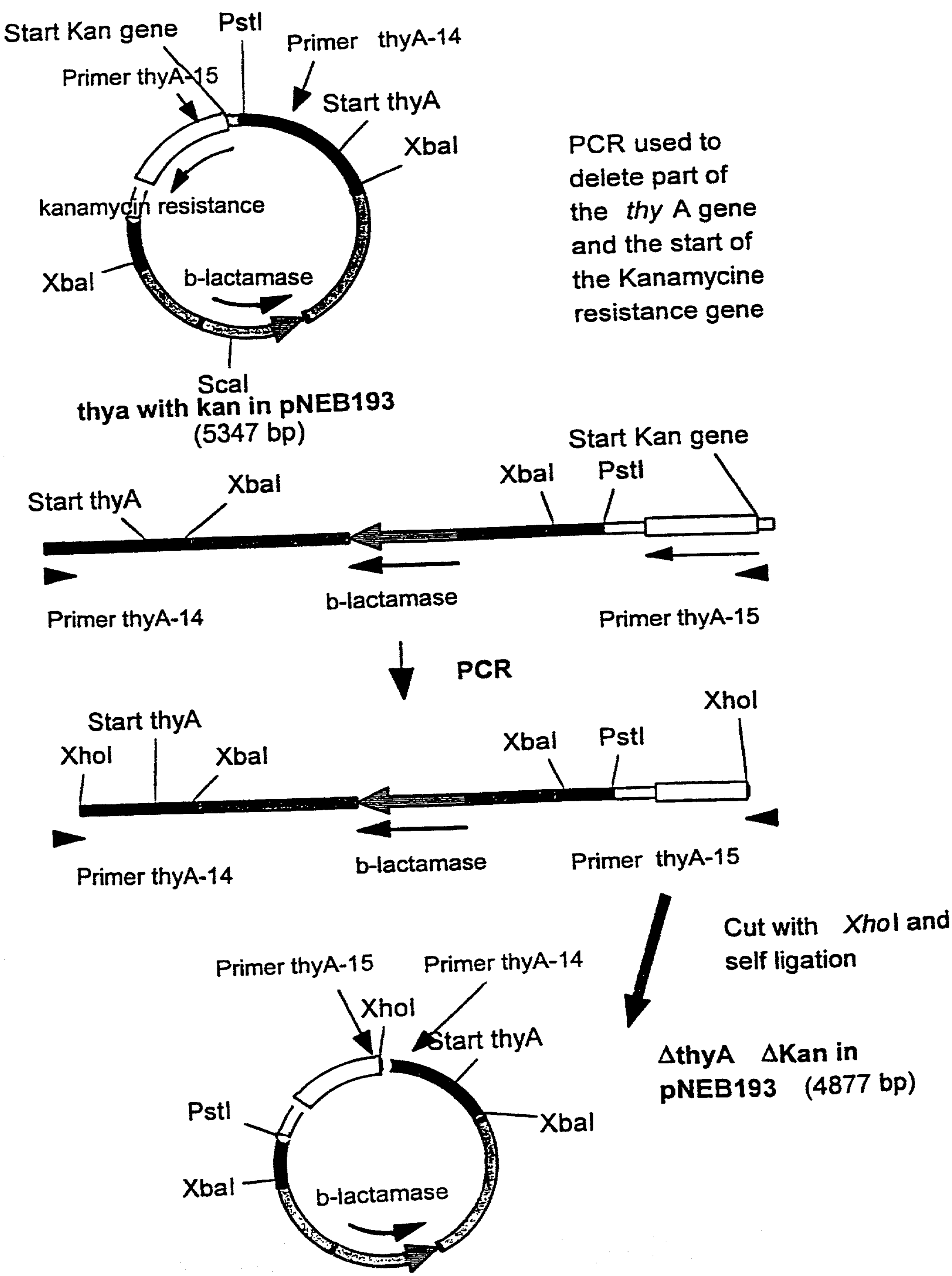
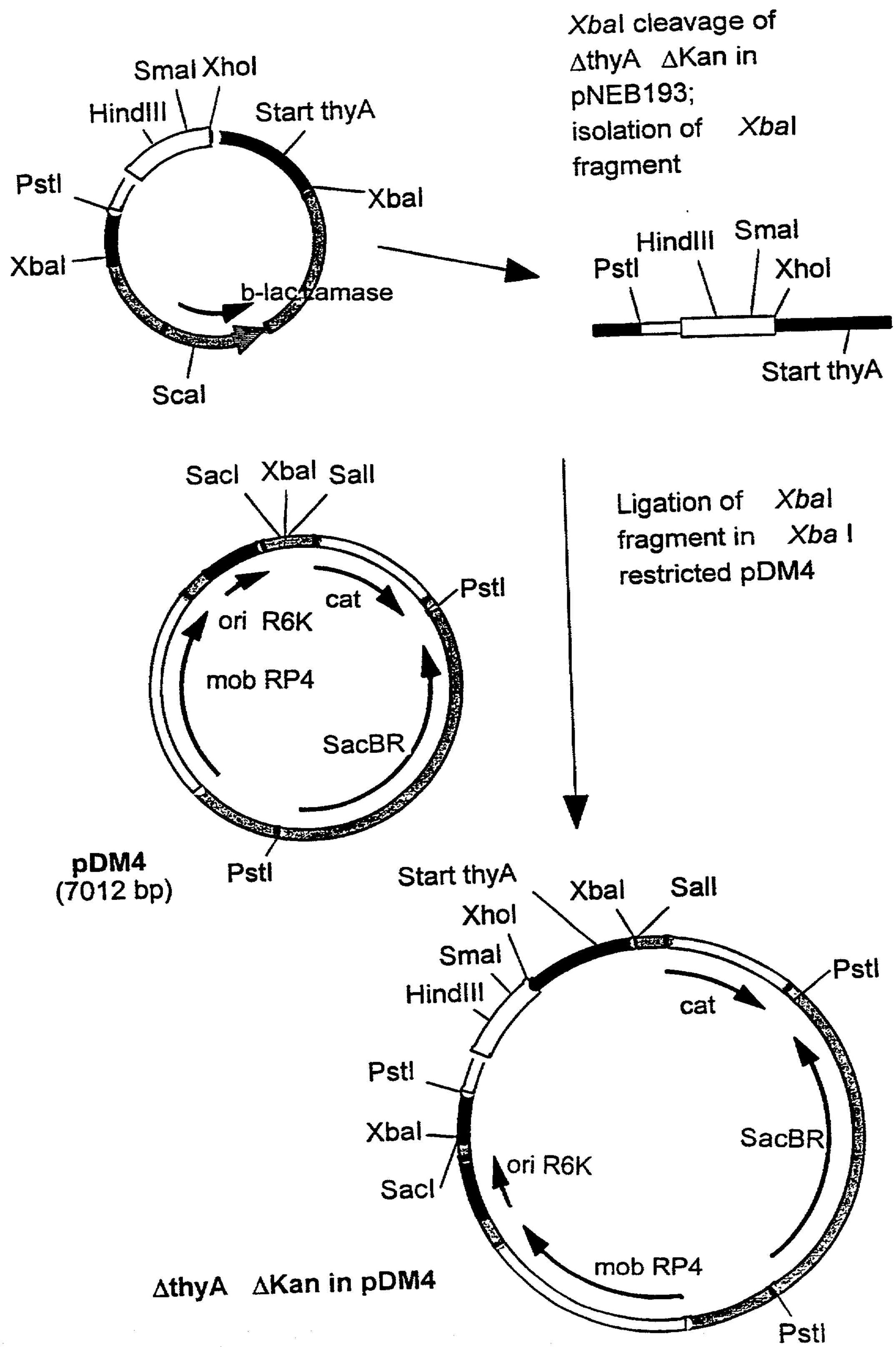

Start Kan gene
PstI
Primer thyA-14
Primer thyA-15
Start thyA
XbaI
kanamycin resistance
XbaI
b-lactamase
ScaI
thya with kan in pNEB193
(5347 bp)
PCR used to delete part of the thy A gene and the start of the Kanamycine resistance gene
Start Kan gene
XbaI
PstI
Start thyA
XbaI
Primer thyA-14
b-lactamase
Primer thyA-15
PCR
XhoI
Start thyA
XhoI
XbaI
XbaI
PstI
Primer thyA-14
b-lactamase
Primer thyA-15
Cut with XhoI and self ligation
Primer thyA-15
Primer thyA-14
XhoI
Start thyA
ΔthyA ΔKan in pNEB193 (4877 bp)
PstI
XbaI
XbaI
b-lactamase

FIG. 12


SmaI XhoI
HindIII
Start thyA
PstI
XbaI
XbaI
b-lactamase
ScaI
XbaI cleavage of ΔthyA ΔKan in pNEB193; isolation of XbaI fragment
HindIII
SmaI
PstI
XhoI
Start thyA
SacI
XbaI
SalI
PstI
cat
ori R6K
mob RP4
SacBR
PstI
pDM4
(7012 bp)
Ligation of XbaI fragment in Xba I restricted pDM4
Start thyA
XbaI
SalI
XhoI
SmaI
HindIII
PstI
cat
PstI
XbaI
SacI
ori R6K
SacBR
mob RP4
PstI
ΔthyA ΔKan in pDM4

09/700712

FIG. 13

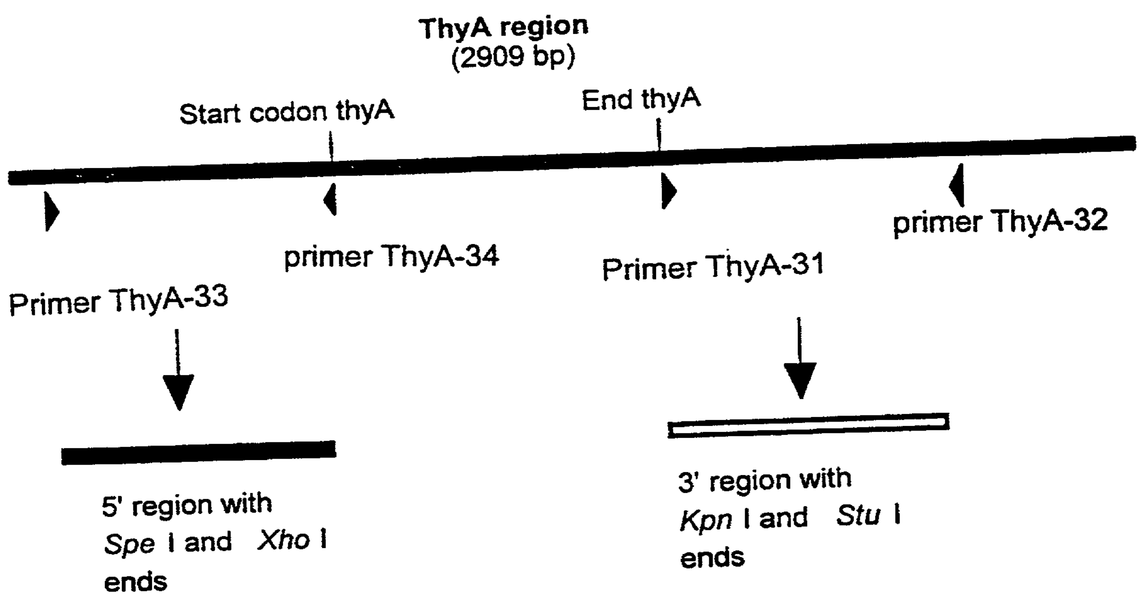


FIG. 14

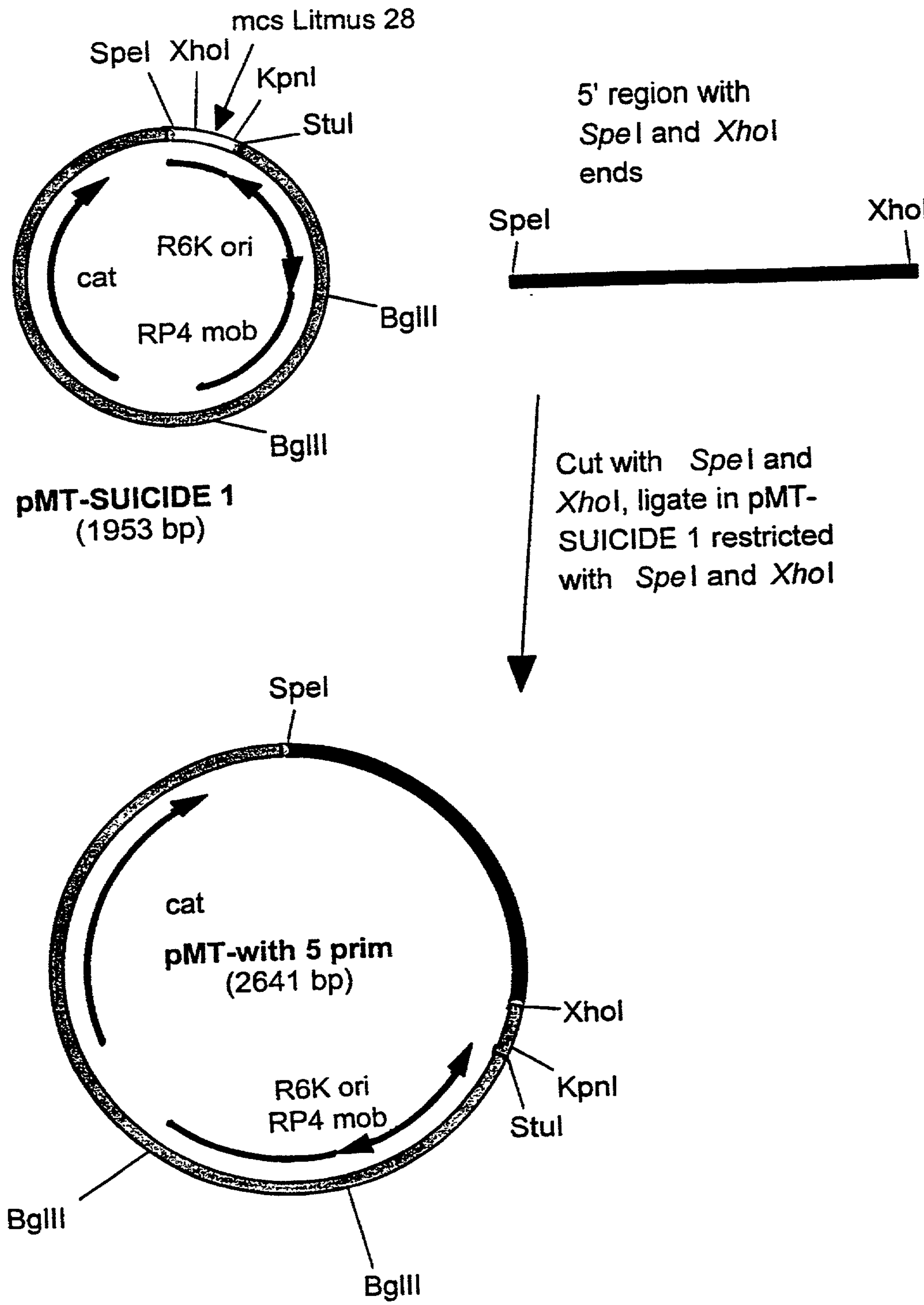
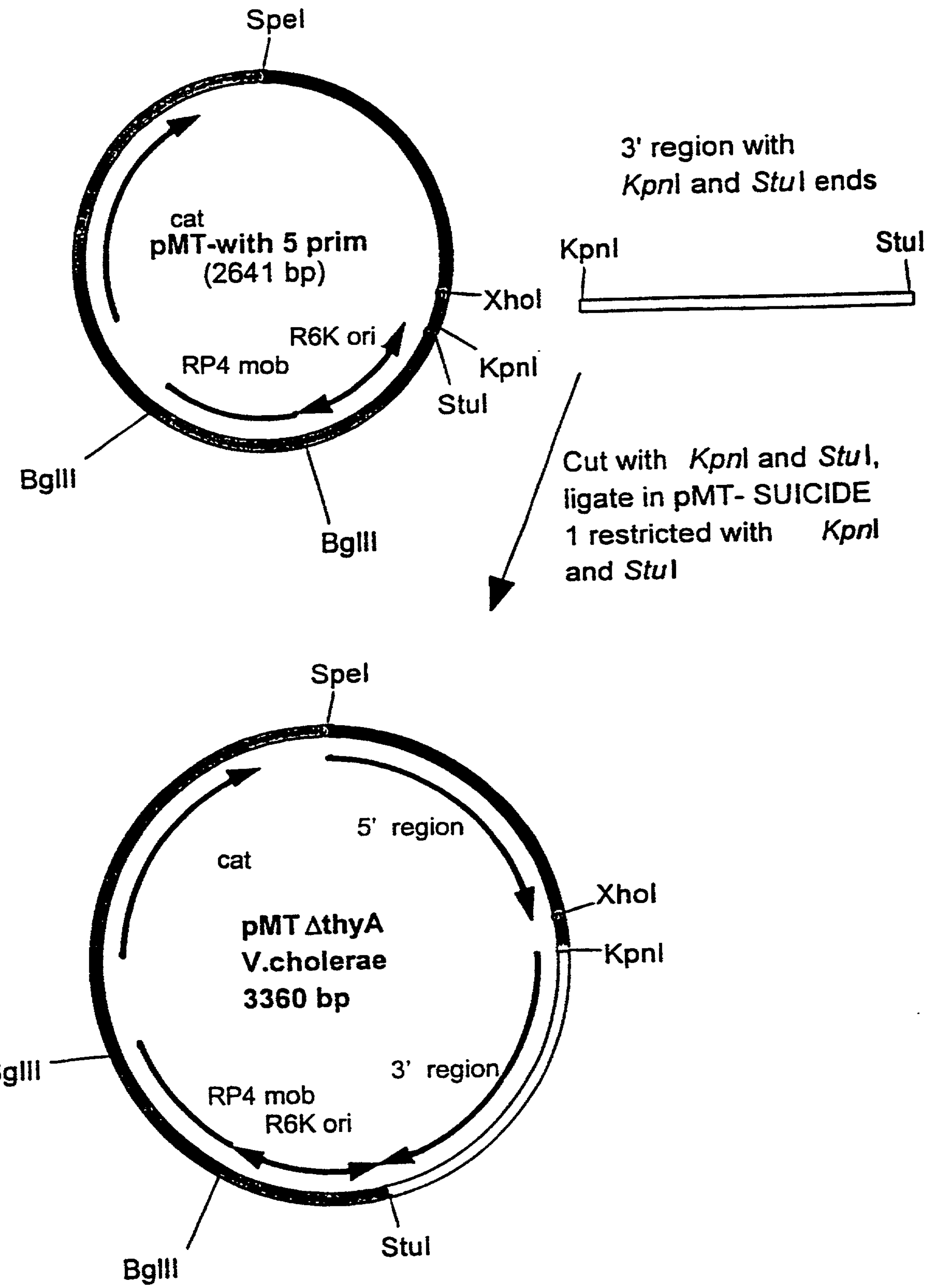

mcs Litmus 28
SpeI
XhoI
KpnI
StuI
cat
R6K ori
RP4 mob
BglII
BglII
pMT-SUICIDE 1
(1953 bp)
5' region with
Spe I and *Xho* I
ends
SpeI
XhoI
Cut with *Spe* I and *Xho* I, ligate in pMT-SUICIDE 1 restricted with *Spe* I and *Xho* I
SpeI
cat
pMT-with 5 prim
(2641 bp)
XhoI
KpnI
StuI
R6K ori
RP4 mob
BglII
BglII

FIG. 15


SpeI
cat
pMT-with 5 prim
(2641 bp)
XhoI
R6K ori
RP4 mob
KpnI
StuI
BglII
BglII
3' region with
KpnI and StuI ends
KpnI
StuI
Cut with KpnI and StuI,
ligate in pMT- SUICIDE
1 restricted with KpnI
and StuI
SpeI
5' region
cat
XhoI
pMT ΔthyA
V.cholerae
3360 bp
KpnI
BglII
3' region
RP4 mob
R6K ori
StuI
BglII

09/700712

FIG. 16

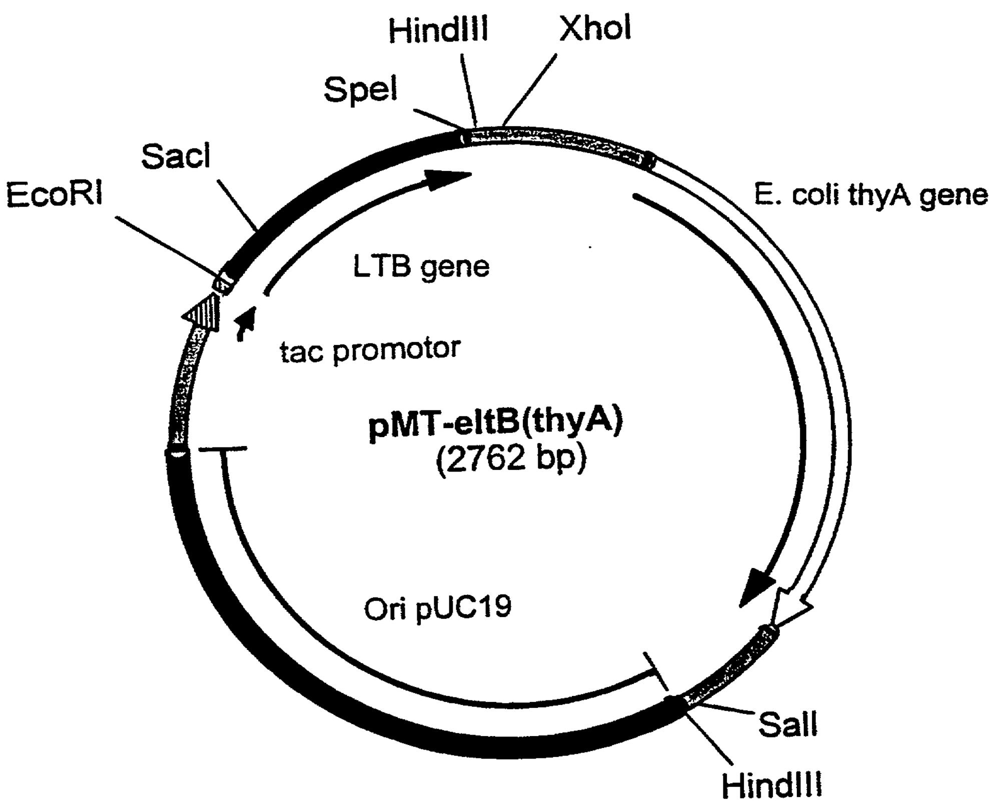
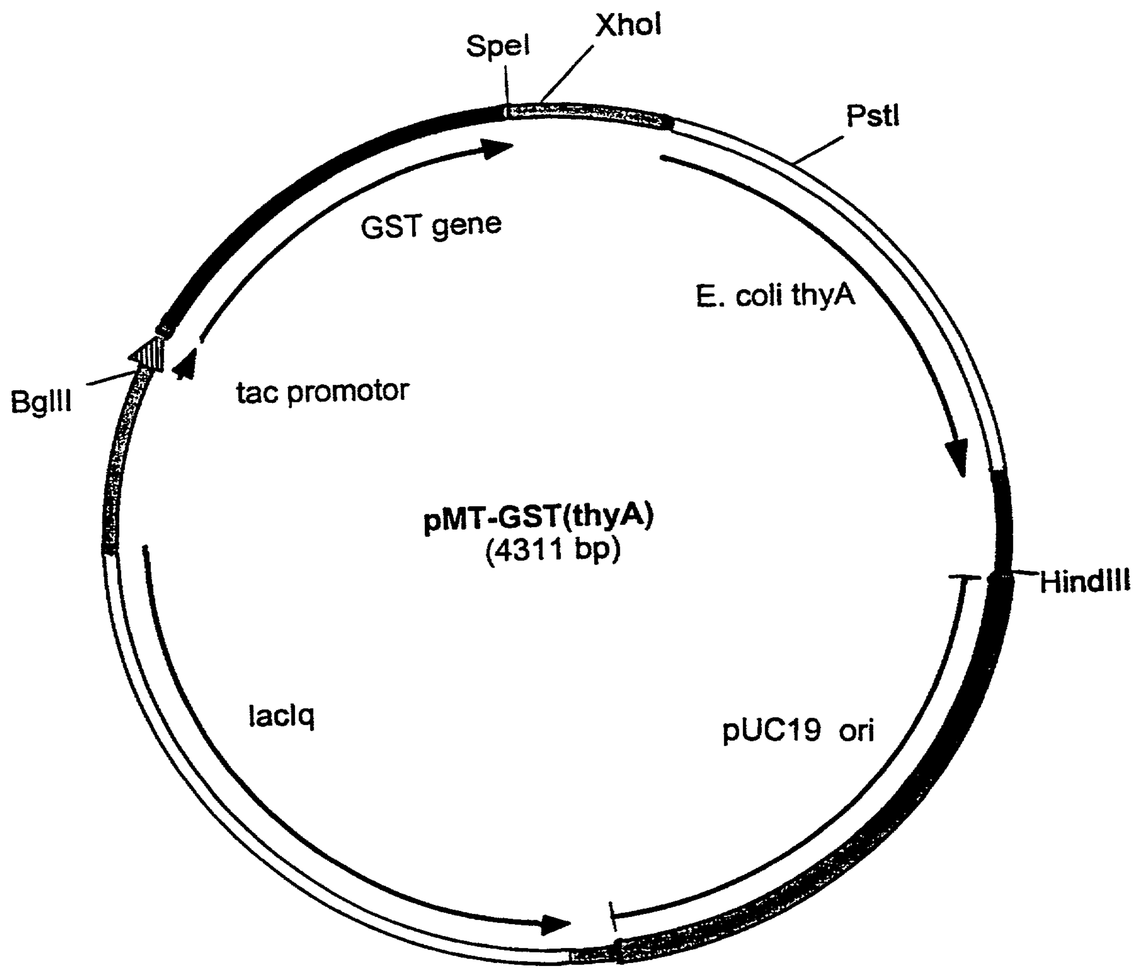


FIG. 17


SpeI
XhoI
PstI
GST gene
E. coli thyA
BglII
tac promotor
pMT-GST(thyA)
(4311 bp)
HindIII
lacIq
pUC19 ori